

**R. Bpu10I $\alpha$** 

MGVEQEWIKNITDMYQSPELIPSHASNLLHQLKREKRNEKLKKALEIITP 50  
 NYISYISILLNNHNMTRKEIVILVDALNEYMNTLRHPSVKSVFESHQADFY 100  
 SSVLPEFFNLLFRNLIKGLNEKIKVNSQKDIIIDCIFDPYNEGRVVFKKK 150  
 RVDVAIILKNKFVFNVEISDFAIPLVAIEEIKTNLDKNMLSGIEQSVDSL 200  
 KETFPCLCYICITELADFAIEKQNYASTHIDEVFILRKQKRGPVRRGTPL 250  
 EVVHADLILEVVEQVGEHLSKFKDPIKTLKARMTEGYLIKKGKG 294

**R. Bpu10I $\beta$** 

MTQIDLSNTKHGSILFEKQKNVKEKYLQQAYKHYLYFRRSIDGLEITNDE 50  
 AIFKLTQAANNYRDNVLYLFESRPNSGQEAFRYTILEEFFYHLFKDLVKK 100  
 KFNQEPSSIVMGKANSYVSLSFSPESFLGLYENPIPYIHTKDQDFVLGCA 150  
 VDLKISPKNELNKENETEIVVPVIAIEECKTYIERNMLDSCAATASRLKAA 200  
 MPYCLYIVASEYMKMDQAYPELTDIDEVFILCKASVGERTALKKKGLPPH 250  
 KLDENLMVELFHMVERHLNRVWWSPEALSRRGRVIGRP 288

**FIGURE 1**

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Subunit	Mutation*	Cleavage dsDNA	of Nicking activity
<i>Bpu</i> 10I $\alpha$	D171A	+++	-
<i>Bpu</i> 10I $\alpha$	E180A	++	+
<i>Bpu</i> 10I $\alpha$	E180Q	+	++
<i>Bpu</i> 10I $\beta$	E168A	+++	-
<i>Bpu</i> 10I $\beta$	E177A	-	+++
<i>Bpu</i> 10I $\beta$	E177Q	+	+++
<i>Bpu</i> 10I $\beta$	K179	-	-

FIGURE 2

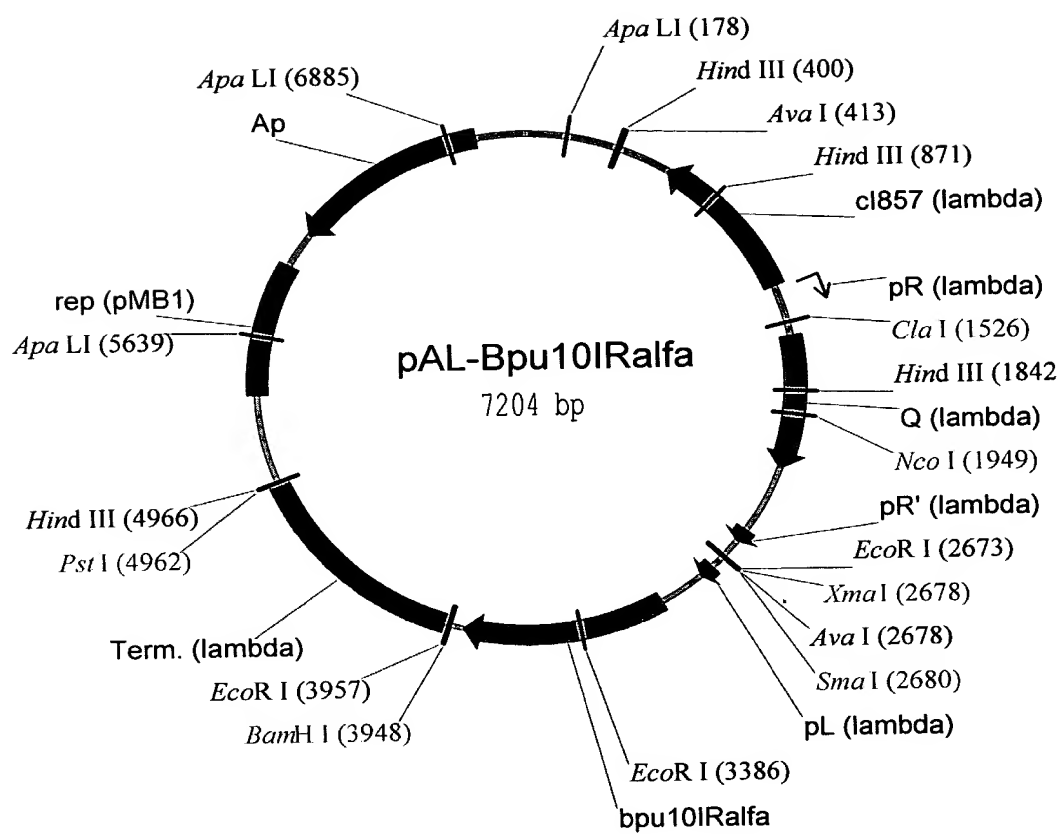


FIGURE 3

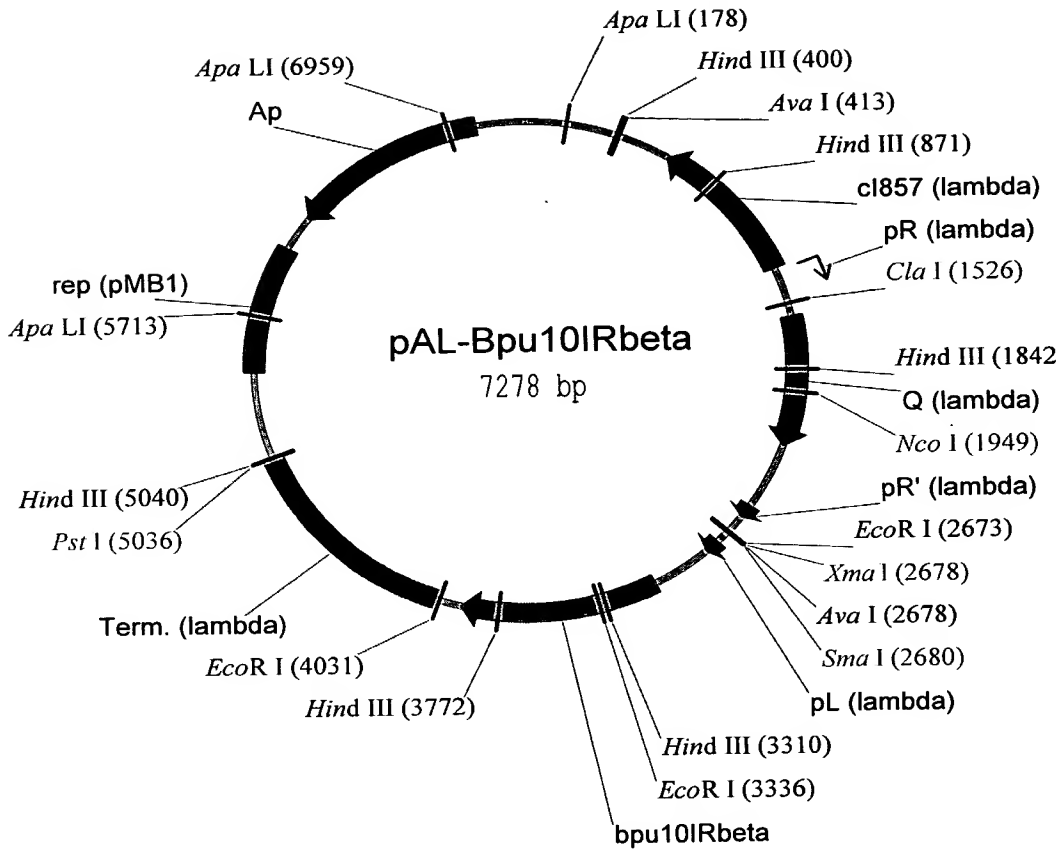


FIGURE 4

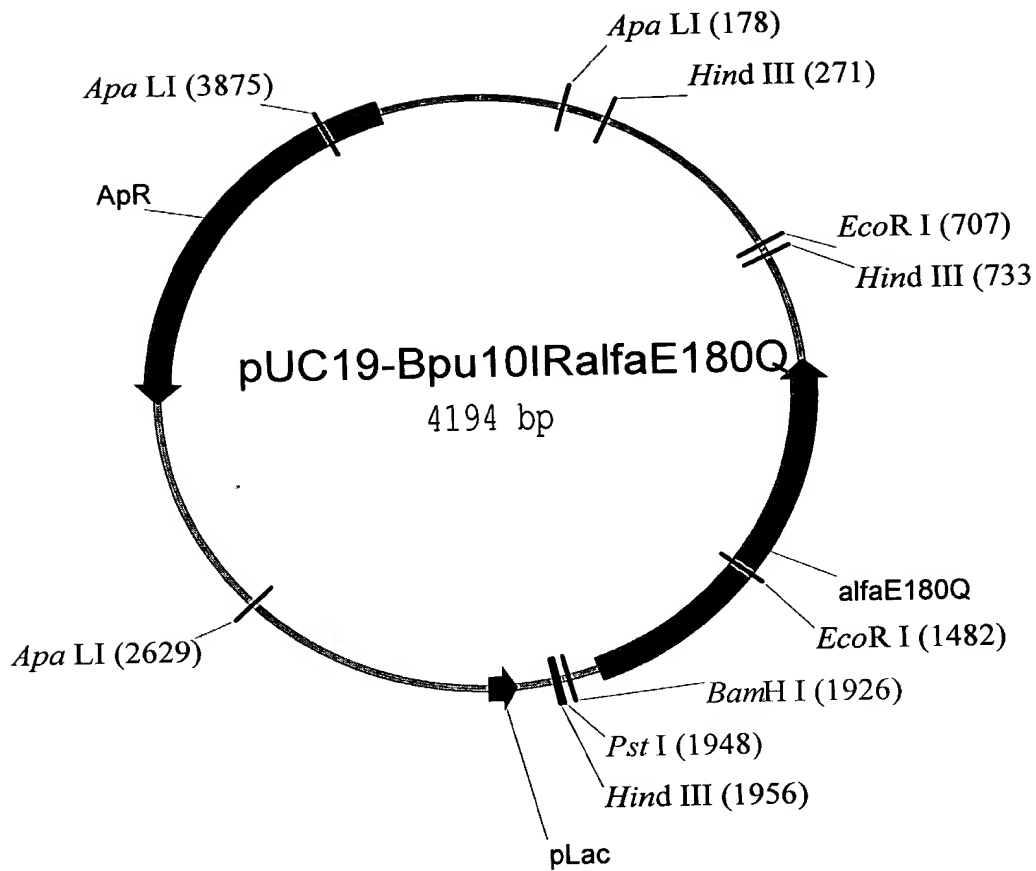


FIGURE 5

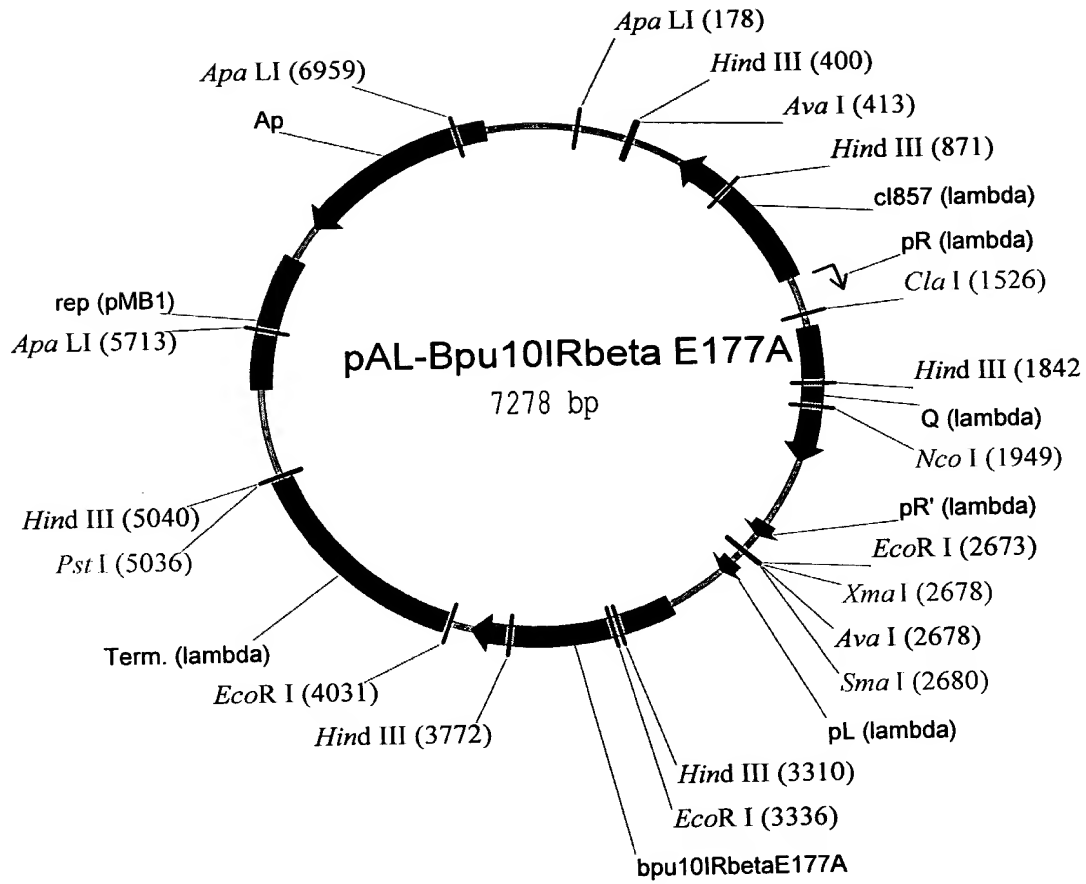


FIGURE 6

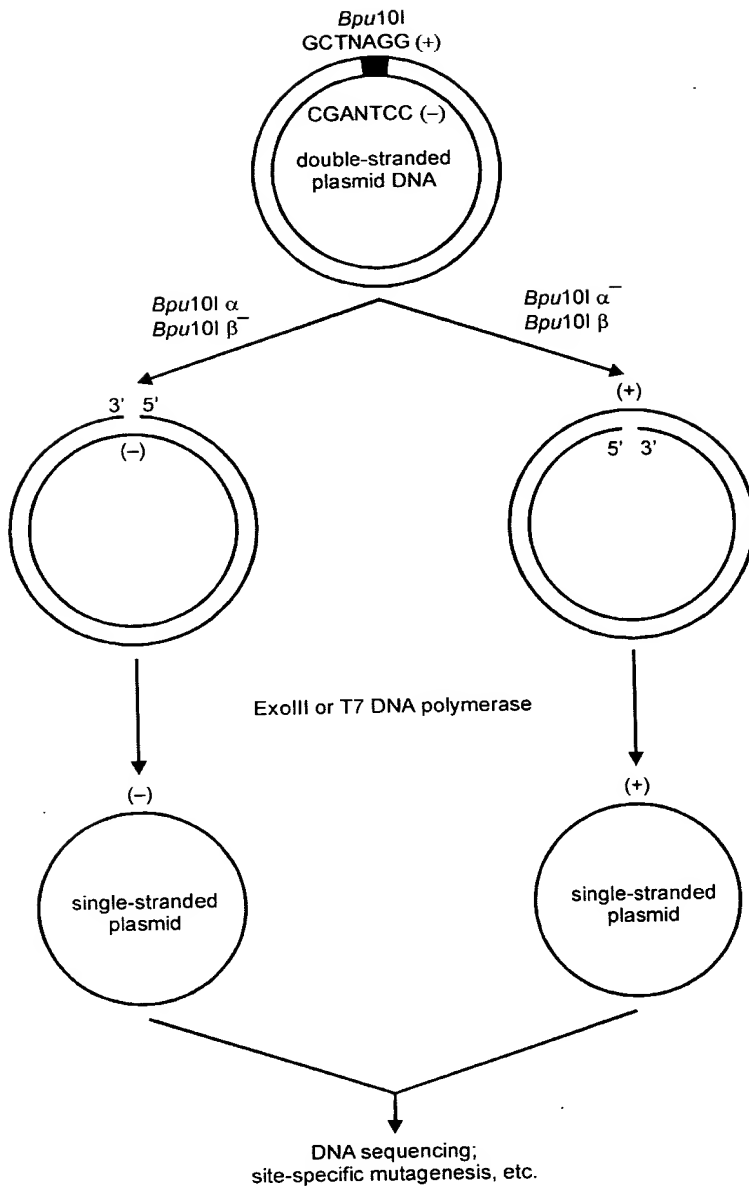


FIGURE 7

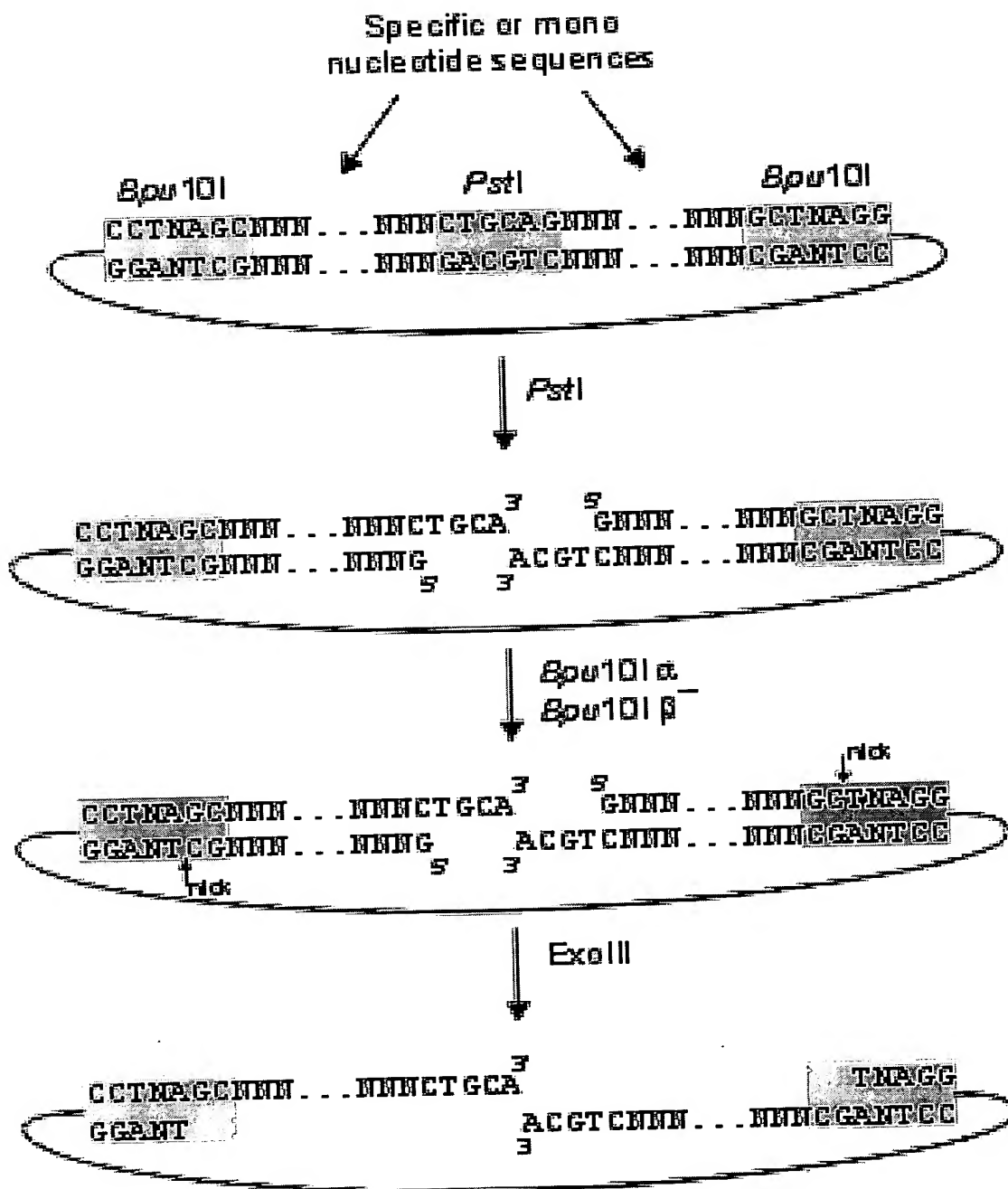


FIGURE 8



↓ 5 min. 95°C, ligation

**FIGURE 9**

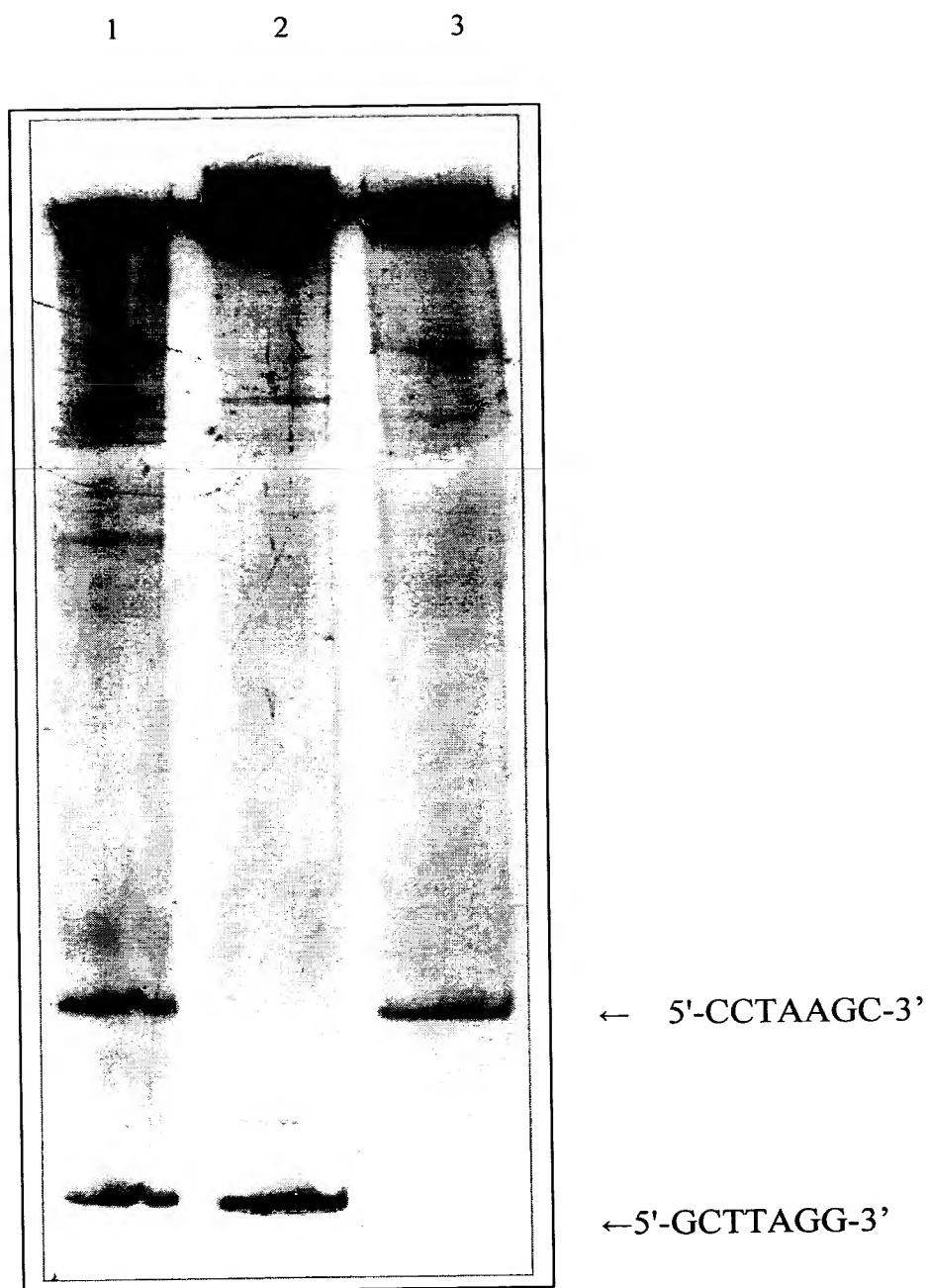


FIGURE 10

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1 2 3 4 5 6 7 8 9 10

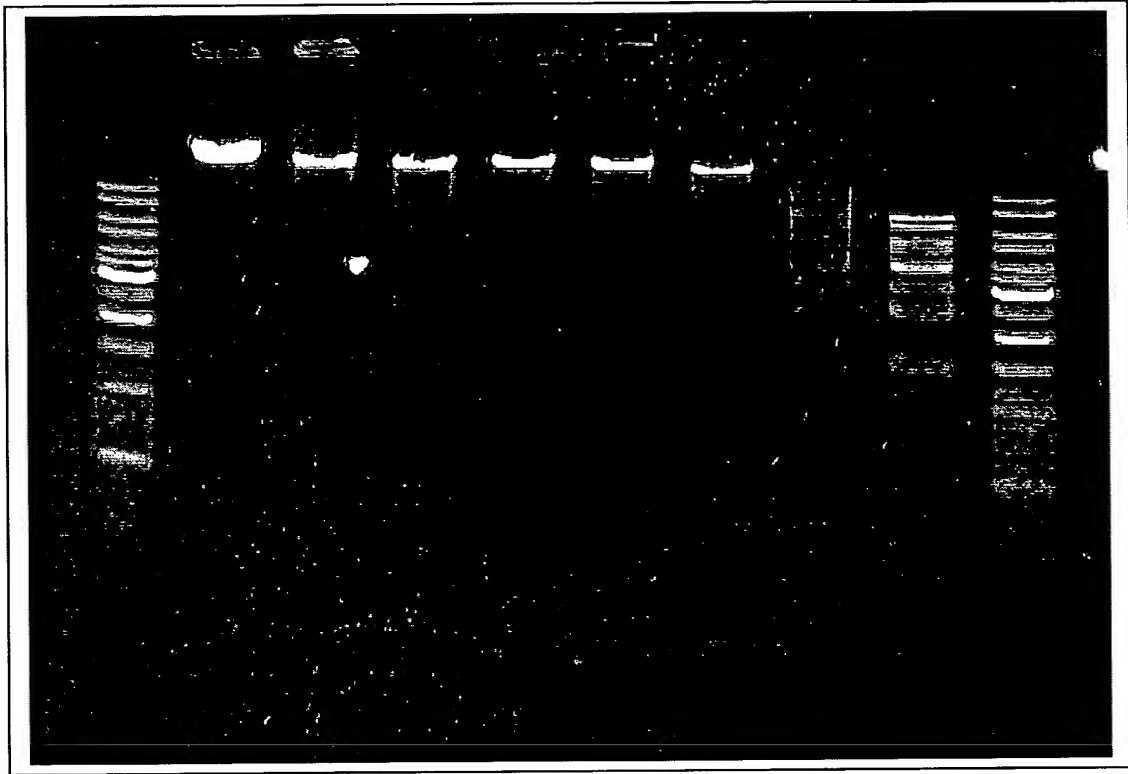


FIGURE 11